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(71) Applicant: **INDENA S.p.A.**  
**Via Ripamonti, 99**  
**I-20141 Milano(IT)**

(72) Inventor: **Bombardelli, Ezio**  
**Via Ripamonti 99**  
**I-20141 Milano(IT)**

(74) Representative: **Bianchetti, Giuseppe**  
**Studio Consulenza Brevettuale Via Rossini,**  
**8**  
**I-20122 Milan(IT)**

(54) **Compositions for treating fat deposits in humans.**

(57) Pharmaceutical and cosmetic compositions for the treatment of superfluous fat deposits contain (a) vegetable active principles having adenylate cyclase agonistic activity or (b) vegetable active principles having antiphosphodiesterase activity or a combination of the two kinds of active principles (a) and (b).

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## COMPOSITIONS FOR TREATING FAT DEPOSITS IN HUMANS

The present invention concerns compositions for treating fat deposits in humans.

More particularly, the invention relates to compositions containing (a) vegetable active principles having adenylate cyclase agonistic activity or (b) vegetable active principles having antiphosphodiesterase activity or a combination of the two kinds of active principles (a) and (b).

5 Obesity is a problem affecting a major and increasing part of western population. The weight reduction in obese subjects may be achieved either by pharmacological means or by selected therapeutic diets: however, in both cases, the more or less dramatic weight reduction does not always give satisfactory results from the aesthetic point of view since the fat deposits in some body areas (particularly thighs, gluteus, hips, knees) remain unchanged, causing unpleasant physical disproportion with consequent psychological dis-  
10 tress of the patient.

The problem of fat deposits effects also some part of the adult female population having normal weight.

In most cases, the pharmaceutical or cosmetic compositions presently available for the treatment of fat deposits do not give the expected results.

It has now been surprisingly found that compositions containing plant extracts or their selected  
15 constituents having agonist action on adenylate cyclase and/or extracts or compounds possessing anti-phosphodiesterase activity, accelerate and make the reduction of fatty deposits located in the above cited areas easier thus obtaining significant advantages both in the elimination of physiological aesthetic defects and in the consequence of the forced body weight reduction.

According to the invention, suitable adenylate cyclase agonists are extracts of plants belonging to the  
20 Convolvulaceae family, such as Ipomea hederacea, Ipomea parassitica, Ipomea batata etc. or belonging to Labiatae family such as Salvia officinalis, Salvia meliorrhiza and Rosmarinus officinalis. As far as the antiphosphodiesterase activity is concerned, useful plants are those containing dimeric flavons derived from apigenine skeleton; among these, Ginkgo biloba, Sequoia sempervirens, Taxus baccata, Selaginella rupestris etc. can be used as a source of active material. The extracts of these plants or their particular  
25 fractions can be used alone or in a mixture in different ratios with adenylate cyclase agonists or with other active principles already used for the same purposes, such as yohimibine isomers.

It is particularly preferred the use of apigenine dimers or corresponding derivatives such as amentoflavone, bilobetine, sciadopitine, ginkgonetine etc., or flavone dimers having similar structure. It is known that amentoflavone is a powerful inhibitor of phosphodiesterase and other dimers having similar structure  
30 also share the same activity.

It has now been found that the combination of these flavones in pure form or of extracts containing them with adenylate cyclase agonists can dramatically decrease fat deposits when topically applied on areas where fats accumulate.

The previously cited Ipomea or Labiatae species extracts are particularly active as adenylate cyclase  
35 agonists.

The adenylate cyclase agonistic activity of Ipomea hederacea, Ipomea batata, Ipomea parassitica, Salvia officinalis, Salvia meliorrhiza is shown in Table 1.

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Table 1.

Adenylate cyclase activity of various crude extracts on bovine heart homogenates.		
Substances	Conc., mg/100ul	Specific Activity
Control	//	21.8 + 3.9
Ipomea hederacea	0.2	51.3 + 4.1
	0.4	58.6 + 0.8
Ipomea parassitica	0.2	32.8 + 2.6
	0.4	33.7 + 1.5
Ipomea batata	0.2	44.6 + 3.9
	0.4	56.3 + 4.6
Salvia meliorrh.	0.2	50.3 + 5.4
	0.4	57.2 + 4.8

The determination of the agonist action of these extracts on adenylate-cyclase was determined according to the method of Kurokawa et al. (Biochim. Biophys. Acta 583,467,1979) using brain, heart homogenates or tumoral cells. Adenylate cyclase activity was assayed on the basis of formation of (3H) cyclic AMP formation from the (3H) ATP. Activity ratio was expressed as a percentage of the activity with the sample against the basal activity. As a reference substance, Forskoline, a well known adenylate-cyclase agonist, has been used.

The determination of the phosphodiesterase activity was carried out according to the method of Beretz et al. (Experientia, 34,1054,1977). The method consists in measuring (3H) products resulting from incubation of (3H)-c-AMP with the phosphodiesterase preparation and with an excess of nucleotides. An anion exchange resin was used in a batch process to remove the residual substrate. The inhibition of PDE by flavonoids was studied in experiments in which the concentration of c-AMP was held constant at 1  $\mu$ M. The I-50 (concentration of flavonoid resulting in a 50% inhibition of c-AMP breakdown) was calculated by interpolating 2 values of inhibition ranging from 35 to 75% against the logarithm of the dose of the flavonoid added. The I-50 values of the most important dimeric flavonoids in this test are reported in Table II.

Substances	I <sub>50</sub>	
	Cyclic AMP	Cyclic GMP
Amentoflavone	0.65	0.58
Ginkgonetine	0.88	0.49
Ginkgo/Flav.	2.46	1.85
Papaverine	5.00	12.44
Cromoglycate Na	2000	235

Assays were performed with 1  $\mu$ M substrate, I<sub>50</sub> was calculated as described above.

In the treatment of undesirable aesthetical body areas the combinations of the hydroalcoholic extracts of *Ipomea hederacea* with the extracts of *Ginkgo biloba* enriched in dimeric flavons or the extracts of *Salvia meliorrhiza* with the extracts of *Ginkgo biloba* or *Selaginella rupestris* were particularly interesting. To reduce fat deposits, the treatment with the above cited extracts must be continued for a few days or weeks depending on the seriousness of the unattractiveness.

The dosage of the extracts or of the pure active constituents of the plants having adenylate-cyclase agonistic or antiphosphodiesterase action or alfa-2-adrenergic receptor blocking action such as  $\alpha$ -yohimbine, may vary between 0.1 mg to 0.2 mg; these products in form of extracts or pure constituents may be incorporated in formulations containing the usual excipients, such as those reported on the Remington's Pharmaceutical Sciences Handbook, Mack Pub. Co., N.Y., U.S.A.. Particularly preferred excipients are both

natural or synthetic phospholipids which increase the absorption of the active principles through the skin. The daily posology of the active ingredients in the formulations depends on the patient's conditions and on the seriousness of the pathology and can be divided in two or more administrations per day. Normally a complete cycle of therapy takes, in combination with a proper diet, a treatment of 10 to 180 days.

5 The active principles of the compositions of the invention are prepared by extracting the vegetable material with alcohols or aliphatic ketones either alone or in admixture with water or, when the active principles are lipophilic, with aromatic and/or aliphatic hydrocarbons or with ethyl acetate. The obtained extracts may be purified from inert or undesired substances by partitioning the active principles between an aqueous phase and selective solvents. An alternative process for the purification of said extracts comprises  
10 the absorption on resins when the extracts are obtained by aqueous extraction or with water miscible solvents.

The absorbed active principles may be selectively released from the resin using solvents with different polarity or high dielectric constant. The resins used for these separations are usually those having polystyrene matrix, such as Amberlites<sup>(R)</sup> and Duolites<sup>(R)</sup>.

15 Duolite S-730<sup>R</sup> and Amberlite XAD2<sup>R</sup> are preferably used.

According to the latter process, the active principles of Salvia miltiorrhiza and of other plants belonging to the Labiatae family may be selectively eluted using toluene/ethyl acetate mixtures preferably in the 8:2 ratio; the active principles of Ipomea species are preferably extracted from aqueous solutions after acidification at pH around 1.5 with n-butanol or mixture of n-butanol and toluene; the extracts obtained  
20 according to the above cited procedures can be directly incorporated in cosmetic or pharmaceutical formulations and used in human medication.

As far as the anti-phosphodiesterase principles are concerned, the methods employed for their preparation are those normally used for dimeric flavons.

The following examples are given by way of illustration of the present invention.

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#### EXAMPLE 1

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##### Preparation of Ipomea hederacea extract.

100 g of Ipomea hederacea seeds are ground and extracted several times with 400 ml of a mixture of ethanol/water 6:4 (v/v); to eliminate drug impurities and flocculated materials, the combined extracts is  
35 filtered and then concentrated under vacuum to dryness; 14 g of brown solid were obtained which are ready for biological application.

#### EXAMPLE 2

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##### Preparation of Salvia miltiorrhiza purified extract.

500 g of Salvia miltiorrhiza roots are ground and extracted 5 times with 2 l of a mixture of ethanol/water 1:1; to eliminate drug impurities the combined extracts are filtered and then concentrated under vacuum to 500 ml (extract/drug ratio 1:1 v/w); the cloudy suspension is extracted four times with 500 ml of ethylacetate; the organic phase after filtration on Na<sub>2</sub>SO<sub>4</sub> is concentrated under vacuum to dryness. 2 g of a red extract enriched in tanshinone like substance were obtained; this purified extract, poorly soluble in water  
50 can be incorporated in oily emulsions alone for the treatment of fatty unattractiveness.

#### EXAMPLE 3

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##### Preparation of purified extract of Salvia officinalis.

500 g of leaves and stems of Salvia officinalis are steam distilled to remove the essential oils; after distillation the drug is continuously extracted with alcohol until exhaustion. The solvent is concentrated under vacuum to 500 ml and the aqueous residue extracted twice with 300 ml of ethyl acetate and the organic phase concentrated to dryness. The residue containing chlorophyll and other inert substances was solubilized in alcohol extracted with n-hexane; the alcoholic phase after concentration to dryness represents the active fraction of Salvia officinalis.

#### EXAMPLE 4

Preparation of dimeric flavonoids mixture from Ginkgo biloba leaves.

500 g of green Ginkgo biloba leaves are extracted with methanol until exhaustion; the solvent is concentrated to small volume and the residue diluted with 100 ml of a mixture of methanol/water 1:1, this phase is extracted twice with 100 ml of chloroform. The chloroform solution is evaporated under vacuum to dryness and the residue, solubilized in 100 ml of methanol/water 60% is extracted with n-hexane. The hexane extract is eliminated while the aqueous methanol is concentrated to dryness. This residue contains about 40% of dimeric flavonoids with amentoflavone skeleton and can be used as an active ingredient in formulations useful in fat deposits reduction.

#### EXAMPLE 5

Preparation of a cream containing as active ingredients Salvia metiorrhiza extract, Ginkgo biloba dimeric flavonoids fraction and alfa-Yohimbine.

Each 100 g cream contains:

Salvia metiorrhiza extract (example 2)	0.2 g
Ginkgo biloba dimeric flavonoids (example 4)	0.2 g
Alfa-Yohimbine	0.05 g
Soybean Phospholipids	2.00 g
Carboxyvinylpolymer (Carbomer 934)	1.2 g
Sodium laurylsarcosinate	0.5 g
Polysorbate 80	3 g
Hydrogenated lanoline	5 g
Spermaceti	5 g
Polyisoprene	5 g
Weat Oil	2 g
Dimethylsilicone oil	0.5 g
Triton 80	0.1 g
Parfum	0.3 g
10% Sodium hydroxide sol	2 g
Water	q.s.100 g

#### Claims

1. Pharmaceutical and cosmetic compositions for the treatment of superfluous fat deposits containing:  
(a) vegetable active principles having adenylate cyclase agonistic activity or (b) vegetable active principles having antiphosphodiesterase activity or a combination of the two kinds of active principles (a) and (b).

2. Compositions according to claim 1 wherein the active principles of a) type comprise Ipomea hederacea, Ipomea parassitica, Ipomea batata, Salvia officinalis, Salvia meliorrhiza or Rosmarinus officinalis extracts.
3. Compositions according to claim 1 wherein the active principles of b) type comprise Ginkgo biloba, Sequoia sempervirens, Taxus bacata, Selaginella rupestris extracts.
- 5 4. Compositions according to claim 1 wherein the active principles of b) type comprise apigenine, amentoflavone, bilobetine, sciadopitisine, ginkgonetine or similar flavone dimers.
5. Compositions according to any one of the previous claims containing alpha-yohimbine.
6. Compositions according to claims 1 or 5 containing Salvia meliorrhiza extracts.
7. Compositions according to any one of the previous claims containing natural or synthetic phospholipid
- 10 mixtures as excipients.

Claims for the following Contracting States: GR, ES

- 15 1. A process for the preparation of pharmaceutical and cosmetic compositions characterized in that (a) vegetable active principles having adenylate cyclase agonistic activity or (b) vegetable active principles having antiphosphodiesterase activity or a combination of the two kinds of active principles (a) and (b) are formulated into suitable administration forms.

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**29.04.92 Bulletin 92/18**

(71) Applicant: **INDENA S.p.A.**  
**Via Ripamonti, 99**  
**I-20141 Milano(IT)**

(72) Inventor: **Bombardelli, Ezio**  
**Via Ripamonti 99**  
**I-20141 Milano(IT)**

(74) Representative: **Blanchetti, Giuseppe**  
**Studio Consulenza Brevettuale Via Rossini,**  
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## PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention  
shall be considered, for the purposes of subsequent  
proceedings, as the European search report

Application Number

EP 90 12 0068

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	DATABASE WPIL/DERWENT, no. 86-210304; & RO-A-88 525 (INTR. NIVEA COSMET) 30-01-1986 * Abstract *	1-7	A 61 K 35/78 A 61 K 31/475 A 61 K 31/35
X	DATABASE WPIL/DERWENT, no. 89-136220; & JP-A-1 083 022 (LION CORP.) 28-03-1989 * Abstract *	1-7	
X	DATABASE WPIL/DERWENT, no. 83-60598K; & RO-A-80 364 (BIOFARM INTR. MEDICAMENTE) 30-11-1982 * Abstract *	1-7	
A	FR-A-2 576 212 (J. P. SIMARD) * Claim 1; page 3, lines 4-7 *	1-7	
A	BE-A- 892 207 (DE VALCK) * Claim 3 *	1-7	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			A 61 K
INCOMPLETE SEARCH			
<p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims</p> <p>Claims searched completely : Claims searched incompletely : 1-7 Claims not searched : Reason for the limitation of the search:</p> <p>The scope of claim 1 (vegetable principles with adenylate cyclase agonistic / anti-phodiesterase activity) cannot be clearly defined on the basis of the available literature. Therefore the relevant search can only be partial.</p>			
Place of search THE HAGUE		Date of completion of the search 28-10-1991	Examiner GERLI P.F.M.
CATEGORY OF CITED DOCUMENTS			
<p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

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### CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claims:
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

### LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,

namely:

See sheet -B-

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims: 1-7 (partially)

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#### LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,

namely:

1. Claims (1-7, partially) : Pharmaceutical and cosmetic composition for the treatment of superfluous fat deposits containing vegetable active principles having adenylate cyclase agonistic activity (optionally in combination with principles having antiphosphodiesterase activity).
2. Claims (1-7, partially) : Pharmaceutical and cosmetic composition for the treatment of superfluous fat deposits containing vegetable active principles having antiphosphodiesterase activity.

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